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cont'd

chromosome of a chromosome pair of a genome of the plant seeds, and further wherein a second exogene of said pair of exogenes is located on a second chromosome of said chromosome pair of said genome of the plant seeds, said first and said second exogenes being in allelic relationship, such that said first and said second exogenes obligatorily segregate to different gametes, wherein said first or said second exogenes do not encode a recombinase.

56. (Amended) The method of claim 47, wherein said first and said second transcribable polynucleotide sequences encode polypeptides or RNA molecules that cause said offspring to be male sterile and female fertile.

57. (Amended) The method of claim 49, wherein said first and said second transcribable polynucleotide sequences encode polypeptides or RNA molecules that cause said offspring to be male sterile and female fertile.

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-9 and 11-57 are pending in this case. Claims 1-9, 11-46, 48 and 52-54 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 47, 49-51 and 55-57 are examined. Claims 47, 49-51 and 55-57 have been rejected. Claims 47, 49-51 and 55-57 have now been amended.

Claim Objections

The Examiner has objected to claims 47 and 49 for various informalities. Claims 47 and 49 have now been amended to incorporate the corrections kindly suggested by the Examiner.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner states that claims 47 and 49, 56 and 57 remain rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner's rejections are respectfully traversed. Claims 47, 49-51 and 55-57 have now been amended.

In particular, the Examiner states that Applicants arguments in a previous response that the recombination events described by Qin et al. and Golic et al. would not pose a problem in the present invention are not convincing.

As is clearly described in the studies performed by Qin et al and Golic et al., the systems generated thereby utilized the Cre and FLP recombinases to generate instability in eukaryotic chromosomes. Qin et al created a "system for large-scale manipulation of eukaryotic chromosomes in vivo" (Qin et al., Page 1706, abstract) while Golic et al. created a "frequent mosaicism ... at predefined sites in the genome" (Golic et al., page 252, abstract).

In both cases, progenies exhibiting active recombinase activity are selected for since such recombinase activity is required for generating the chromosomal instability sought after by Qin et al and Golic et al.

In sharp contrast, the recombination event employed by the method of the present invention is a transient event, since the recombinase sequence is selected out prior to the generation of the final product (final plant progeny).

As is clearly illustrated by the PowerPoint presentation enclosed herewith, (Appendix A), the present methodology employs recombinases to generate a stable, modified DNA sequence within the genome of the plant. Once recombination occurs, the presence of the recombinase is no longer required, and as such, the gene encoding the recombinase is selected out in a subsequent crossing and selection step (Figures 1,2,3 and 5 on pages 2,3,4 and 10 of the PowerPoint Presentation).

As is clearly illustrated by the results presented in the PowerPoint presentation, such steps of recombinase utilization and negative selection generated plants which include the modified exogenic DNA sequence and yet are devoid of the recombinase encoding DNA (Figures 3,4 and 5 on pages 9,10 and 11 of the PowerPoint presentation). In addition, as illustrated in later steps selected plants inherit the modified exogenic DNA in a stable manner showing no indication of unwanted recombinase events. (Figure 6 on Page 13 of the of the PowerPoint presentation). Contrary to the Examiner's assertion, the present method yielded stable exogenic allclism in all of the plants that received both alleles (Figure 6 on page 13 of the of the PowerPoint presentation). In one specific case, the two original plants that were use for the cross (EC-G+C and EC-G) were heterozygous and not homozygous and therefore some of the plants shown in Figure 6 contain one allele. The studies of Qin et al and Golic et al. utilize an active Cre recombinase in order to forcibly generate recombination between two chromosomes. The recombinase utilized by these studies is always active and therefore, the recombination events are not transient events, but rather are a series of continuous recombination events designed for creating desired instability in selected chromosomal regions.

Since excision events depend upon the presence of an active recombinase (e.g. Cre), and since plants generated according to the teachings of the present invention clearly do not include recombinase sequences no additional excision/insertion events will occur and therefore the plants generated according to the teachings of the present invention will exhibit stable and inherited exogenic allelism.

The Examiner further states that Gidoni et al. teaches that substantial variation occurs in the timing of FLP recombinase activity.

Although Luo et al and Gidoni et al. report low FLP recombinase activity it should be noted that in contrast to the methodology employed by Luo et al. and Gidoni et al., which methodology requires high FLP efficiency for success, the present methodology, which employs transient recombinase activity does not

require high recombinase activity since plant progenies exhibiting desired recombination events can be selected for using well known and routinely employed biochemical and/or molecular methods (as is clearly illustrated in the enclosed presentation).

The system described by Gidoni et al., requires 100% FLP activity since this recombinase is utilized to switch on a silent gene, or to remove an exogenic DNA (transgene) (and restore 100% fertility) from a genome of agriculturally important plants.

The system of hybrid seed production described by Gidoni et al. is based on an introduction of male sterility (by over expression of an RolC gene which is flanked by FRT sequences) to a female line which is pollinated by a second line which expresses an FLP recombinase and is used to restore fertility. Thus, 100% FLP activity is required to restore fertility in the resultant F1 plants of Gidoni et al.

As such, The F1 plants generated by Gidoni et al. must all be a product of the excision event (e.g. fertile). If the efficiency of the FLP recombinase is less than 100%, the F1 generation will include fertile plants (if excision event occurs and the RolC gene is excised) as well as sterile plants (if the RolC gene is not excised).

Since the method of the present invention includes a selection step following the excision event, the generation of a stable line of plants exhibiting exogenic allelism is enabled.

It will be appreciated in this case, that even in the event that FLP-recombinase activity is as described by Gidoni et al. (without the modification suggested by Lou et al or Lloyd and Davis), selection enables isolation and utilization of specific plants which exhibit the desired excision.

Thus, in sharp contrast to the prior art cited herein, the present invention utilizes transient recombinase activity as a tool for obtaining plants characterized by exogenic allelism. Such plants, which represent the product of the present methodology do not require nor do they harbor recombinase sequences and as

such would not be plagued by undesired recombinase events as suggested by the Examiner.

The Examiner further states, with respect to claims 50-51 and 55, that given the lack of a written description in the specification with regards to structural and physical characteristics of the claimed plants, one skilled in the art would not have been in possession of the genus claimed at the time the application was made.

Applicant contends that this statement is in error. As is clearly illustrated in the enclosed presentation, selection of plants harboring specific DNA sequences can be effected easily using well known molecular or biochemical techniques.

For example, Figure 6 (Page 12 of the PowerPoint presentation) of the attached PowerPoint presentation illustrates that a plant characterized by exogenic allelism which was generated according to the teachings of the present invention was readily identifiable using procedures routinely utilized in the art.

In addition, it should be noted that one distinguishing quality of the plants of the present invention is the allelic relationship formed between the two distinct exogenes expressed thereby.

Since such exogenic relationship stipulates that, for example, an expression product of one exogene activates a promoter of the second exogene (e.g., claim 50), one of ordinary skill in the art could easily type the plants of the present invention by simply detecting the presence of both exogenes or their products using standard molecular techniques such as PCR or Southern and Northern blotting or biochemical techniques such as Western blotting.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 47, 49-51 and 55-57 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner's rejections are respectfully traversed. Claims 47, 49-51 and

55-57 have now been amended.

Applicant has elected to adopt the Examiner's suggestions concerning amendments to claims 47, 49-51 and 55-57. However, the phrase "said offspring" of claim 57 has not been amended since sufficient antecedent basis for the term "offspring" is provided in the next to last line of claim 49.

35 U.S.C. § 102(b) -Vergunst et al.

The Examiner has rejected claim 55 under 35 U.S.C. § 102(b) as anticipated by Vergunst et al.

The Examiner states that Vergunst et al. teach plants that have different exogenes in an allelic relationship on two chromosomes of a chromosome pair, and that these genes would inherently segregate to different gametes. The Examiner further states that the construct described by Vergunst et al. is analogous to the construct of Figure 1.

The Examiners rejections are respectfully traversed. Claim 55 has now been amended.

The Examiner states that the *bar* gene on the first chromosome of a chromosome pair, and the *bar* and *nptII* genes on the second chromosome represent the two exogenes of the chromosomal pair and as such are similar to the T7 polymerase and the Toxin genes illustrated in Figure 1 of the instant application.

The trait of the plants of the present invention which stipulates that "said first and said second exogenes obligatorily segregate to different gametes" would not be present in the plants described by Vergunst et al since the integrated DNA is not stable as long as Cre is expressed from one of the chromosomes.

The segregation test performed by Vergunst et al. (Page 2732, right column) was designed for illustrating the stability of the specific integration, however, since Cre is continuously expressed in this system, such a test is not representative of proper trait segregation.

Thus, the plants described by Vergunst would, if at all, display an allelic

relationship for *nptII* and *bar* only as a short-lived event, since the presence of the Cre recombinase will ensure continuous sequence "shuffling", and loss of such an allelic relationship. It should be noted that such sequences shuffling of *nptII* and *bar* implies that the germ line of such plants would not display obligatorily segregation to different gametes of *nptII* and *bar*, as is characteristic of the two exogenes of the plants generated according to the present invention.

The resistant seedling described by Vergunst et al., are seedlings grown following transformation and selection. The original *Arabidopsis* plants transformed with the integrant DNA (Lox-*nptII*) were either hemizygous or homozygous for the target sequence (35S-Lox-cre).

If these original plants were homozygous and site-specific integration occurred in both target lox site (both chromosomes), the resultant resistant seedlings would be homozygous for the functional *nptII* gene and homozygous for the inactive Cre gene; such plants would be irrelevant to this case.

If the integration occurred at only one lox site (one chromosome), the resultant plants would be homozygous for the Cre gene (one copy being immediately downstream of a promoter and as such, functional and the other copy being nonfunctional) and hemizygous for a functional *nptII* gene.

Although in principal it can be said that the plants which result from one integration event are similar to the plants described in Figure 1 (claim 55) of the instant application, a more careful inspection of the study performed by Vergunst et al. clearly reveals that this is not the case.

Vergunst et al. note in the discussion that "...apparently, in cells homozygous for the target allele, stable integration at one of the lox sites was prevented by expression of Cre from the second allele, leading to rapid reversion of integrate events" (page 2733 first paragraph). Since the integrant is unstable it can not be inherited by the next generation and thus, the teachings of Vergunst et al do not anticipated claim 55 of the present invention which essentially defines exogenic allelism as "the allelic positioning of two functionally distinct exogenes on the chromosomes of a chromosome pair such that substantially 100 %

segregation of the two exogenes is observed upon gamete formation".

The statements made by Vergunst et al. lead to the conclusion that none of the plants generated thereby would exhibit substantially 100% segregation of the exogenes upon gamete formation and as, such the plants of the present invention are clearly distinct from the plants described by Vergunst et al.

In addition, it should be noted that in order to generate the plants described thereby, Vergunst et al. must utilize a recombinase as one of the functional genes.

In sharp contrast, the plants of the present invention can include any two functionally distinct genes as the exogenes.

Thus, to further distinct the present invention from the teachings of Vergunst et al. and expedite prosecution of this case, Applicant has elected to amend claim 55 to include the limitation "wherein said first or said second exogenes do not encode a recombinase".

In view of the above arguments and claim amendment it is applicant's strong opinion that the teachings of Vergunst et al. do not anticipate nor do they render obvious claim 55 of the present invention.

35 U.S.C. § 102(b) - Fabijanski et al.

The Examiner states that claim 55 is rejected under 35 U.S.C. § 102(b) as being anticipated by Fabijanski et al.

The Examiner states that Fabijanski et al. teach plants that have two different exogenes in allelic relationship are male sterile and fertile when crossed to a male fertile plant.

The Examiner's rejections are respectfully traversed. Claim 55 has now been amended.

The definition of allelism provided in the instant application limits exogene positioning to precisely same locus of a chromosomes pair. The plants of the present invention exhibit such exogenic allelism and as the exogenes harbored thereby will always segregate to different gametes.

In sharp contrast, Fabijanski et al. do not teach nor do they suggest methodology suitable for generating such exogenic allelism.

In fact, the progeny screening, map analysis or Pulse Field Electrophoresis (PFE) techniques suggested by Fabijanski et al. as suitable methodology for screening transformants of two random and independent transformation events would be useless in uncovering transformants exhibiting exogenic allelism.

As is well known by one of ordinary skill in the art, utilizing such methods for generating the plant claimed by the present invention would require undue experimentation which would in all likelihood never lead to success.

First, the chance that two independent random insertion of T-DNA will occur at the same locus of the chromosome is very low and it depends on the size of the genome. For example, a corn genome is about 2.5×10^9 bp, therefore, using the methods suggested by Fabijanski et al., the statistical probability of generating corn plants exhibiting exogenic allelism would be absurdly low. To generate and screen this number of double transformants would require several skilled laboratory technicians decades even in case where marker assisted screening is utilized. Thus, an ordinarily skilled artisan would not even attempt, let alone consider using such methods for generating plants exhibiting exogenic allelism.

Fabijanski et al. disclose that "segregation of the first and second recombinant DNA molecules occurs during meiosis and the chance of recombination... is substantially reduced". Although Fabijanski et al. anticipate segregation, such segregation was not described in this study since recombination was not abolished but simply reduced.

Thus, although Fabijanski et al. propose generation of plants which are similar to the plants of the present invention, the methodology proposed thereby cannot be realistically used to generate plants exhibiting true exogenic allelism since while theoretically it is conceivable that the method proposed by Fabijanski et al. can be utilized to generate plants exhibiting exogenic allelism, realistically achieving success using this approach is highly improbable.

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As such, it is Applicant's strong opinion that the teachings of Fabijanski et al. do not anticipate nor do they render obvious claim 55 since the male-sterile plants described by Fabijanski et al. would not exhibit obligatory segregation of the exogenes into different gametes, and thus would not exhibit 100% male fertility restoration when out-crossed.

In view of the above amendments and remarks it is respectfully submitted that claims 47, 49-51 and 55-57 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,



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Date: May 30, 2001.

Enclosed:

Three month extension fee

Version with marking to show changes made

Appendix A

Declaration by Vered Yesodi along with attached CV

VERSION WITH MARKING TO SHOW CHANGES MADE**In the claims:**

47. (Thrice Amended) A method of generating exogenic allelism in a plant, the method comprising the steps of:

- (a) providing a first plant and a second plants ~~each including isogenic~~ for an expression cassette in the same chromosomal location, said expression cassette comprising:
 - (i) a first segment comprising a first promoter sequence;
 - (ii) a second segment comprising a first transcribable polynucleotide sequence; and
 - (iii) a third segment comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, said third segment being flanked by said first and second segments, wherein a pair of site-specific recombination sequences are disposed one between said first segment and said third segment and another between said second segment and said third segment, such that said first promoter sequence is operatively coupled with said first transcribable polynucleotide sequence only following excision of said third segment from the expression cassette by site specific recombination via said pair of site-specific recombination sequences;
- (b) introducing a recombinase into said first plant, so as to excise said third segment thereby operatively adjoining said first transcribable polynucleotide sequence to said first promoter sequence;
- (c) selfing a plant ~~resultant~~ resulting from step (b) and selecting a

progeny which is devoid of said recombinase-minus;

- (d) crossing a plant ~~resultant~~ resulting from step (bc) ~~and with~~ said second plant thereby obtaining an offspring characterized by exogenic allelism.

49. (Thrice Amended) A method of generating exogenic allelism in a plant, the method comprising the steps of:

- (a) providing a first plant and a second isogenic plant each including ~~hemizygous or homozygous for~~ an expression cassette in the same chromosomal location, said expression cassette comprising:
- (i) a first segment comprising a first transcribable polynucleotide sequence, said first transcribable polynucleotide sequence being operatively linked to a first promoter sequence, said first segment being flanked by a pair of first site-specific recombination sequences; and
- (ii) a second segment, being linked to said first segment, said second segment comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, said second segment being flanked by a pair of second site-specific recombination sequences;
- (b) introducing a first recombinase into said first plant, so as to excise said first segment, and selfing said first plant and selecting a progeny which is devoid of said first recombinase-minus;
- (c) introducing a second recombinase into said second plant, so as to excise said second segment, and selfing said second plant and selecting a progeny which is devoid of said second recombinase-minus; and
- (d) crossing a plant ~~resultant~~ resulting from step (b) with a plant

~~resultant~~resulting from step (c), so as to generate an offspring characterized by exogenic allelism.

50. (Twice Amended) A plant homozygous for an expression cassette comprising:

- (a) a first segment comprising a first promoter sequence;
- (b) a second segment comprising a first transcribable polynucleotide sequence; and
- (c) a third segment comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, said third segment being flanked by said first and second segments, wherein a pair of site-specific recombination sequences are disposed one between said first segment and said third segment and another between said second segment and said third segment, such that said first promoter sequence is operatively coupled with said first transcribable polynucleotide sequence only following excision of said third segment from the expression cassette by site specific recombination via said pair of site-specific recombination sequences;

said second transcribable sequence ~~being selected such that~~encoding an expression product ~~thereof capable of activates-activating~~ said first promoter sequence to direct transcription of said first transcribable sequence.

51. (Twice Amended) A plant homozygous for an expression cassette comprising:

- (a) a first segment comprising a first transcribable polynucleotide sequence, said first transcribable polynucleotide sequence being operatively linked to a first promoter sequence, said first segment

being flanked by a pair of first site-specific recombination sequences; and

- (b) a second segment, being linked to said first segment, said second segment comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, said second segment being flanked by a pair of second site-specific recombination sequences, said second transcribable polynucleotide sequence ~~being selected such that~~encoding a polypeptide or an RNA~~an expression product molecule thereof regulates~~capable of regulating an expression level of a product of said first transcribable polynucleotide sequence.

55. (Thrice Amended) Plant seeds ~~each of which comprising a genome, said genome~~ comprising a pair of exogenes, wherein a first exogene of said pair of exogenes is located on a first chromosome of a chromosome pair of ~~said a genome of the plant seeds~~, and further wherein a second exogene of said pair of exogenes is located on a second chromosome of said chromosome pair of said genome of the plant seeds, said first and said second exogenes ~~being functionally hemizygous and~~ in allelic relationship, such that said first and said second exogenes obligatorily segregate to different gametes, wherein said first or said second exogenes do not encode a recombinase.

____ 56. (Amended) The method of claim 47, wherein said first and said second transcribable polynucleotide sequences ~~are selected such that~~encode polypeptides or RNA molecules that cause said offspring ~~is to be~~ male sterile and female fertile.

57. (Amended) The method of claim 49, wherein said first and said second transcribable polynucleotide sequences encode polypeptides or RNA

molecules that cause~~are selected such that~~ said offspring is to be male sterile and female fertile.